A REËXAMINATION OF THE CYTOLOGY OF HYDRACTINIA AND PENNARIA.[1]

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The present report does not claim to be a complete discussion of the cytological phenomena in the developmental history of *Hydractinia* and *Pennaria*, nevertheless, it adds considerable new data hitherto unpublished concerning the problem of the structure of the egg, the migration of the chromatin, maturation, fertilization and mitosis. However, the present paper cannot claim to settle the controversy as to the existence of amitosis because the results concerning which there may be some differences of opinion have been negatively interpreted.

During the summer of 1906 Professor C. W. Hargitt asked me to undertake a reëxamination of some of his work on hydroids paying particular attention to the cytological phenomena of the early development, that being the point of most interest. The results of Bunting, '94, on *Hydractinia* were not in agreement with his observations on *Pennaria*, *Eudendrium*, *Clava*, etc., so that he suggested that it might be well to restudy this form also. While I have had opportunity to examine all of Professor Hargitt's preparations on the several hydroids studied by him, it seems wise to confine this paper to *Hydractinia* and *Pennaria*.

It is not often that such a study as this is undertaken and when it is there is involved a great deal of work that it would be superfluous to republish so that the present paper needs to be read in connection with Bunting, '94, on *Hydractinia* and Hargitt, '04, on *Pennaria*. At the beginning of this study, Professor Hargitt explicitly stated that he wished to give me absolute freedom in the problem and the interpretation of the results. This he has done even to the extent of not seeing any of my preparations until I turned over the finished paper to him.

When he asked me to undertake this restudy, he volunteered to collect and preserve the material necessary. In this particular

[1] Contributions from the Zoölogical Laboratory, Syracuse University, C. W. Hargitt, director.

too much credit cannot be given Professor Hargitt for his persistent experiments in trying to find a suitable fixing reagent for these refractory eggs of hydroids. Had I been without the benefit of his long experience, I doubt if the present results could have been secured. He used, among fixing agents, Bouin's fluid which has given the best fixation of any thus far tried and certainly these preparations are superior in regard to their fixation to any which he made during the preceding years of study. There is very little doubt that the eggs of hydroids degenerate when left in alcohol for some time and should be embedded in paraffin as soon as possible.

It seems unnecessary to review the general literature on this subject for this has already been well done by Hargitt in his numerous papers and by Bigelow, '08. Professor Hargitt is so well known as an authority on hydroids that it has seemed unnecessary for me to make an elaborate critique of his papers. Therefore, I have in most instances simply referred to the pages where he discussed similar phenomena, elaborating only those points upon which I have additional data.

Origin of the Eggs in *Hydractinia.*

The eggs arise in the entoderm close to the basement membrane. Certain entoderm cells are directly transformed without any immediately preceding cell division. These young ova are distinguished by having a large nucleus and granular cytoplasm. No special region in the polyp is devoted to the production of ova as Bunting, '94, maintains and text-figures 1 and 2 demonstrate. The young ova are as likely to begin their growth in the cells in the base of the polyp as in those along the side.

Growth of the Egg in *Hydractinia.*

The first apparent change in these entoderm cells which are to become ova that has thus far been observed is a marked increase in the size of the nucleus before the cell as a whole has undergone any change. The result is that the nucleus occupies most of the cell, the cytoplasm being limited to a narrow border. A comparison of the nuclear contents at this early stage with the surrounding entoderm cells shows that the nucleolus is increas-

ing rapidly in size. In the entoderm cells it is so very small that it is difficult to be sure of its presence in some cells, and the same is true in some of the young ova, but in most instances it is a clearly defined and easily distinguished body.

The most interesting change is found in the chromatin network. In the young ova it is now in its most conspicuous state. From

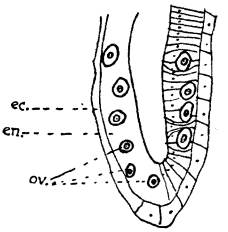

FIG. 1. Outline drawing of base of polyp to show the position of the ova. *ec*, ectoderm; *en*, entoderm; *ov*, ova. *Hydractinia*.

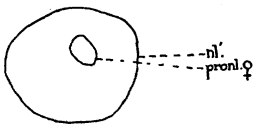

FIG. 3. Camera lucida drawing of the egg nucleus, the larger circle, and the female pronucleus, the smaller circle. *Nl*, nucleus; pronl. ♀, female pronucleus.

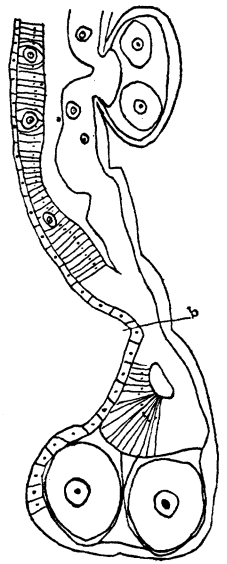

FIG. 2. Outline drawing to show that sometimes the Hydractinian polyp branches. *B*, base of polyp. Reconstructed from several sections.

this time on until new ova arise in a new polyp the chromatin does not possess such distinctness. A loose network is distributed through the nucleus with conspicuous masses where the threads cross. The achromatic substance (residual substance of Lillie, '06) does not stain in the acid or basic stains. The nuclear membrane is very distinct, chiefly due to the fact that a considerable amount of chromatin material seems to be directly in contact with it. The cytoplasm is as yet free from the microsomes — minute granules — which characterize the slightly older ova.

The next step in this gradual transformation is the rapid increase in the cytoplasm, accompanied by a growth in the nucleus. The cytoplasm is now loosely sprinkled with microsomes, in

places giving the appearance of a reticulum. With the accumulation of the microsomes, the reticulate condition is obliterated and the cytoplasm becomes a dense mass of microsomes. In such young ova the achromatic substance of the nucleus takes a plasma stain such as Orange G or Bordeaux red. The vacuolation of the nucleolus has begun by this time, the vacuoles taking the plasma stain. This vacuolation of the nucleolus is in agreement with the many cases already described. The vacuolation continues until the nucleolus disappears which occurs before maturation. The growth, vacuolation and eventual disappearance of the nucleolus is an event which takes place during the growth of the egg, but is not synchronous with any definite phase of this growth.

The young ova thus described are found in the entoderm at the base, the side of the polyp, or in the gonophore. About this time the ova take their permanent position (Bunting) in the gonophore, *i. e.*, in the ectoderm where they increase greatly in size. This increase in size is largely a matter associated with the growth of the individual microsomes into small spherules. We are led to believe from this study that there are also many microsomes added which likewise are changed into spherules. These spherules are so numerous in the adult egg as to conceal the ultimate structure of the cytoplasm. They take a dense stain but not always a homogeneous one. During this gradual growth, the staining reaction changes, a change which can be readily seen on a slide where all stages are represented. A number of such were studied where all of the material had received the same treatment from fixation to and including staining. On such a slide the young ova are so deeply stained as to conceal most of the details of structure while the large eggs show but a faint response to the stain. But when the more mature eggs are once stained in iron hæmatoxylin, it takes considerable time to differentiate them in the iron so tenaciously do they hold the stain. Under these conditions one may see on the same slide young ova black and blue-black in color while the older eggs are hardly stained at all. Between these two extremes, there are all gradations. This change in color reaction is evidently due to the transformation of the microsomes into spherules.

Chromatin Changes in the Egg of *Hydractinia*.

While the cytoplasm is becoming thus transformed, the nucleus has increased in size although it does not become more distinct. The nucleolus is mostly occupied by a large vacuole. In place of the sharply defined chromatin masses in the younger ova, there is a marked change in this particular. The typical chromatin reaction is hardly evident and the whole nucleus tends to take a stain similar to the cytoplasm. The chromatin threads and masses are less definite in position and arrangement. The question naturally arises, what is becoming of the chromatin? Can its disappearance be traced into any part of the egg? In other words, is there a definite and specific migration of chromatin from the nucleus into the cytoplasm? The following facts are submitted in answer to these queries.

While the eggs are still in the gonophore and the nucleolus is becoming vacuolated, small particles of chromatin leave the nucleus and wander out into the cytoplasm. Fig. 1, Pl. I., shows the early stages of this process, some of the chromatin granules are just emerging. Others have moved some distance. My attention was first directed to this phenomenon by finding eggs which showed conditions such as Fig. 2, Pl. I., typifies. A number of densely staining chromatin granules are scattered in the cytoplasm and mostly surrounded by a narrow clear area. These masses of chromatin are small and usually single but occasionally two or three are found in a single vacuole. The reason for regarding these masses as chromatin is because they give the same color reaction as similar shaped bodies in the nucleus; and for the further reason which is obvious in Fig. 1, namely, the actual migration of the chromatin from the nucleus.

When the size of the nucleus of the mature egg, *i. e.*, before maturation, is compared with the female pro-nucleus one is strongly impressed with the great reduction in size. Text-fig. 3 is a camera lucida drawing of the outline of the egg nucleus as represented by the larger circle, while the smaller circle within represents the size of the female pro-nucleus. It must be apparent at once that up to and during maturation there is a remarkable reduction in the size of the nucleus which the maturation phenomenon alone does not adequately explain. The nucleus

loses a large amount of chromatin by direct migration into the cytoplasm which is entirely independent of the chromatin discharged during the formation of the polar bodies. The subsequent fate of this discharged chromatin has been studied with much pains and it is the belief of the writer that it is something as follows: When the cytoplasm of the egg is examined after it has been discharged from the gonophore there appear many areas that are free from the characteristic granules of the surrounding cytoplasm. These areas are usually round and contain small particles that stain with Borax carmine or hæmatoxylin. They look so much like faintly staining nuclei that their appearance is very confusing at first (Fig. 6). As segmentation progresses, these areas tend to migrate to the periphery of the egg and are occasionally so numerous that they form a nearly continuous row around the embryo, Fig. 9. Eventually they are absorbed by the cytoplasm. This explanation, then, traces the highly vacuolated condition of the cytoplasm in *Hydractinia* directly to the migration of chromatin from the nucleus before maturation begins. A similar series of changes occurs in *Pennaria* but at a different time.

Localization of the Formative Stuffs in *Hydractinia*.

The following extract indicates the extent of the previous description of the structure of the cytoplasm. "Sections of the egg show deutoplasm spheres distributed throughout the protoplasm, with the exception of the outer rim which is composed entirely of protoplasm" (Bunting, page 215).

The cytoplasm exhibits a rather definite localization of the so-called formative stuffs in the presence of a coarsely granular crescentic area (picro-acetic fixation) located on the side of the egg in which the nucleus lies—the animal pole. The appearance of these granules in *Hydractinia* is very similar to what Hargitt, '06, p. 214, has found in *Clava*. In addition to these bodies there are some minute bodies located around the periphery of the egg in a narrow band which takes a deep blue stain (Borax carmine, Lyons blue method). These particles do not stain readily and so were overlooked by Bunting. By the regular hæmotoxylin methods they are usually indistinguishable from the other micro-

somes and spherules. This gives three distinct bodies in the cytoplasm: (1) The ordinary bodies all through the cytoplasm and usually interpreted as yolk masses; (2) the coarse granules distributed in crescentic bands; (3) the small bodies around the periphery. The small peripheral granules remain on the outside of the embryo during cleavage and can be traced into the planula. In the planula they are confined to the ectoderm. The differentiation and subsequent fate of similar particles has been made out in *Pennaria*. The first and second class of granules are chiefly confined to the mass of cells within the ectoderm of the planula, although a few scattering granules are seen in the ectoderm.

Maturation in *Hydractinia*.

The following quotation reveals the extent of previous observations on this phase of development. "The ovum while in the gonophore has a well-defined nucleus situated above the center of the egg, which fades from view when the ovum is deposited" (Bunting, page 215). "In about fifteen minutes after the ova are laid the polar bodies appear. When first observed two globules were present, one had been extruded, while the other one was just appearing. One of the two divided subsequently, in a plane at right angles to the first cleavage plane of the ovum. Within ten minutes from the extrusion of the first polar body, the second was ejected" (Bunting, page 216).

The nucleus during growth varies from round to elliptical and in this latter shape the ends may nearly reach to the periphery but until maturation begins, the nucleus is central in position. It is to be regretted that more stages in maturation have not been discovered notwithstanding the fact that large numbers of slides have been made of eggs just after deposition. Maturation begins before the eggs are discharged from the gonophore but just how long I am unable to state. The fact that this process begins while the eggs are still retained, makes the solving of the problem tedious in as much as the gonophores are not set free as are the medusæ in *Pennaria*. Occasionally a gonophore containing eggs is found among the recently discharged eggs and it was in such that the first signs of maturation were detected. A large number of slides were made of the large gonophores from colonies that

were laying eggs when fixed but none of these showed any of the maturation phenomena. Fig. 3, Pl. I., shows the prophase of the first maturation. The asters have moved part way around the nucleus and a few spindle fibers are evident. The nuclear wall on the side toward the middle has been partly broken down. The chromatin shows but a slight tendency to take a stain although it is collecting into definite masses. The chief importance of this drawing centers around the process by means of which maturation occurs, namely, the mitotic process.

When this material was collected it was not thought that maturation began until after deposition because of the observations quoted. For that reason much time was spent studying the eggs just after deposition, but in all cases none of the earlier stages were found. In two or three eggs undoubted polar bodies were found after the eggs had been deposited, and such a case is shown in Fig. 4, Pl. I. Fig. 4 represents the late telophase of what I judge to be the second maturation. In the polar cell there are several vesicles which probably represent chromatin. In the egg the chromatin vessels are very small and grouped among the remains of the breaking down astral fibers. These vesicles collect into a single vesicle, the female pro-nucleus, Fig. 5, Pl. I.

Certainly these two figures do not furnish a complete account of maturation, but they do show: (1) The nature of the process; (2) that this process begins in the gonophore; (3) that Miss Bunting's observations are not correct, because if what she observed were polar bodies they would be found attached to the egg before and after segmentation, but such is not the case. The polar bodies usually drop off as soon as the eggs are discharged. In all of the eggs studied but two were found which retained the egg nucleus after the egg had been deposited.

One of the curious conditions is shown in Fig. 5, where the female pro-nucleus lies next to the periphery of the egg with a few fibers apparently starting from its outer pole. This was thought for some time to mean the prophase in maturation, but never could fibers or an aster be found at the inner pole. Just why there should be a small furrow over this nucleus I do not know, and such a furrow does not always occur. It might be

thought to mean the beginning of segmentation, but such a furrow may appear before fertilization takes place. In a number of instances the nucleus in this same stage was seen to be partly protruding from the substance of the cytoplasm, a condition for which no explanation is offered. The chromatin in the female pro-nucleus takes a faint stain up to the time of the first cleavage. The differentiation of the egg of *Hydractinia* is very difficult, much more so than in *Pennaria*, which makes the recognition of these very arduous. In the region of the nucleus in Fig. 5 there are several deeply staining particles which look much like chromatin, but of their nature I am uncertain. That this nucleus is the female pro-nucleus the following reasons indicate: (1) The absence of the nucleolus; (2) its relatively small size; (3) that it has been traced directly into the first cleavage.

Fertilization in *Hydractinia*.

Thus far no sperms have been found in the eggs before they were deposited but after deposition spermatozoa are seen in contact with the eggs. The sperm head becomes transformed into a vesicle soon after it penetrates the cytoplasm. There does not seem to be any definite place where the sperm enters the egg. During the progress of the male pro-nucleus through the cytoplasm, no aster was seen nor any definite path. The staining reaction of this body is so very faint that it is made out only after careful study with the oil immersion.

Fig. 6, Pl. I., shows the approach of the male pro-nucleus and the change in shape of the female pro-nucleus preparatory to the prophase of the first segmentation. No asters or radiations could be distinguished in connection with either of these pro-nuclei at this stage. These observations further show that the egg nucleus does not "fade from view when the ovum is deposited" but that it can be traced continuously from the egg in the gonophore to the first segmentation stage.

Cleavage in *Hydractinia*.

The difficulties encountered in differentiating these eggs has made it almost impossible to discover a complete series of the changes in any of the stages as many of the mitotic phenomena

are visible only with the oil immersion lens. This means that but few eggs will be cut in just the right plane to enable one to make out the correct relations ; and it also means that important conditions will escape detection.

The first division of the egg into the two-cell stage is preceded by the formation of a definite mitotic figure after the male and female pro-nuclei have come together. The chromatin becomes more responsive to stain and gradually condenses into a definite number of very minute chromosomes. These chromosomes in size and staining reaction are so similar to some of the granules in the cytoplasm, Fig. 8, Pl. II., that it is impossible to be certain that they are chromosomes unless the spindle fibers are present. This makes the determination of the number of chromosomes difficult because when one has a cross-section of the metaphase through the equatorial region, one cannot determine the relation of the spindle fibers to the chromosomes and so cannot be certain of their number. The task was a little easier in *Pennaria*, where fourteen were counted in the anaphase, although here I am not certain that this is the correct number. I think that there are from twelve to sixteen chromosomes present in these hydroids, the exact number remains to be determined.

The chromosomes form in the typical metaphase condition, split and move toward each pole of the spindle. In the anaphase distinct interzonal fibers are present. During the telophase the chromosomes are transformed into a nucleus. This nucleus does not necessarily assume the rounded outline but is often elongated and even irregular in shape. The prophase of the next cleavage frequently begins while the nucleus is in this condition, Fig. 7, Pl. I. In Fig. 7 a typical prophase of mitosis in cleavage is shown. The faint asters are on opposite sides of the elongate nucleus, and a few spindle fibers are forming preparatory to the metaphase and the dissolution of the nucleus. The centrosphere as shown in Figs. 7 and 8 will be discussed in a separate section.

A definite mitotic figure has been made out in all of the early stages of segmentation and followed up to the planula stage. A typical condition of the early embryo is shown in outline in Fig. 9, Pl. II. The cells surround a cavity which is first seen in the two-cell stage and is due to the separation of these first two cells.

This cavity increases in extent as segmentation continues. After a time the peripheral cells begin to segment in such a manner as to set cells free in this cavity. The direction of the spindle in Fig. 8 shows the method. Fig. 8 was taken from one of the cells shown in Fig. 9 and in each of the remaining blastomeres there is a mitotic figure, so placed as to give rise to a cell that becomes free in the segmentation cavity. The segmentation cavity finally becomes full of cells due to the setting free of cells from the periphery and the subsequent division of these same cells as they lie in this cavity.

Membranes in *Pennaria*.

First or False Membrane. — The absence of a membrane or membrane-like structure in the animal egg is doubted by some notwithstanding that Wilson, '82, Metschnikoff, '86, Hargitt, '04, and others have repeatedly stated that the eggs of hydroids are naked. Brauer, '91, finds in *Hydra*, after the embryo is formed, two membranes produced by the ectoderm, but the unsegmented egg is naked. Previous studies in *Pennaria* make no mention of an egg membrane, but the ectosarcal portion of the egg is described by Hargitt as forming at times a filamentous membrane around the cytoplasmic papillæ. Aside from these references no mention is made of membrane in hydroids.

When the eggs of *Pennaria* are well fixed in Bouin, and a hæmatoxylin stain is followed by a plasma stain such as Bordeaux red, a rather broad but uneven structure is readily observed. It first forms while the egg is still growing and is easily made out while the eggs are still in the medusa. The portion of the egg adjacent to the manubrium shows pseudopodia-like processes (cf. Hargitt) and between these processes this structure is quite wide and irregular in width. After the eggs are deposited and have assumed the rounded outline, this membrane-like structure appears as shown in text-fig. 4. It is rarely of uniform thickness and usually shows one place that is bulging. Also

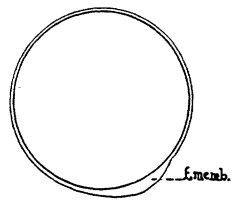

Fig. 4. Outline drawing to show the relative thickness and variability in width of the false membrane. *F. memb*, false membrane.

in the laid eggs as segmentation begins and continues, it appears torn so that some of the eggs will be only partly surrounded by it. Some time during the progress of cleavage this membrane disappears entirely. It is well known that the newly deposited eggs of *Pennaria* are inclined to stick to bits of grass, a glass dish, etc., which is due to the adhesive property of the false membrane. The term false membrane is applied to this structure because it is not permanent; and the term membrane is used because it seems to serve the purpose and do the work of a real membrane. No differential lamellæ were visible in this false membrane either before or after deposition. Numerous sperm heads are frequently to be distinguished within its substance. The inequalities in thickness of the false membrane suggest that this substance is of a fluid nature in the living egg and transparent. If this interpretation prevails, then one can no longer speak of the eggs in *Pennaria* as being naked.

Second or True Membrane. — After fertilization and with the beginning of cleavage a second membrane begins to form (cf. Hargitt) which lies in close contact with the granules of the cytoplasm and completely surrounds each cell as it is produced in cleavage. This structure compares favorably in appearance, staining reaction, etc., with the regularly described egg membranes of animal eggs and so is designated as the true membrane.

Maturation of the Egg in *Pennaria.*

From the early development of the egg up to the beginning of maturation I cannot add any new data, but do confirm Hargitt's ('04, p. 456) observations. The extensive observations of Hargitt as well as my present independent studies indicate that it is very rare to find a polar body attached to the egg after deposition, not one in one hundred will show a polar body at this time. It is also very rare to find a deposited egg that still retains the egg nucleus with a nucleolus. In fact this latter structure, the nucleolus, has been taken as an indication of whether maturation has taken place or not. Where it is lacking I have designated the nucleus as the female pro-nucleus. The egg nucleus in *Pennaria* does not show any such great size as is found in *Hydractinia* and varies but slightly from the female pro-nucleus which is

always present in the egg previous to the beginning of segmentation.

Sections of medusæ after they have become free from the colony and before the eggs are discharged may show the presence of polar bodies. Several different slides show conditions such as are indicated in Fig. 10, Pl. II. The first polar body is being shoved to one side by the formation of the second. The chromatin is in the form of three vesicles in the first polar body. Even while the egg is in the medusa, the polar body may be pushed some distance from the spot where it emerged which may be due to the contractions of the bell of the medusa. The second polar body has ten vesicles in two groups; while thirteen are made out deeper in the egg. To the right there is one isolated vesicle. This one and several of the thirteen contain granules of chromatin. This interpretation of the vesicles grows out of my

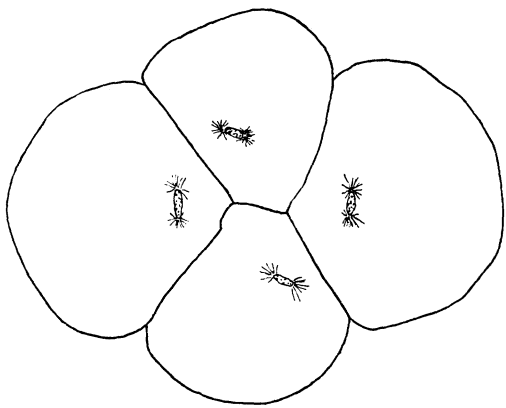

FIG. 5. A regular segmentation stage, all cells in prophase.

study of the changes through which the chromatin passes during cleavage. The several vesicles deep in the egg unite into a single definite nucleus like the one in Fig. 11, Pl. II. That these polar bodies are formed by the mitotic process, there is but little doubt because of similar conditions in *Hydractinia* where there can be no doubt as to the beginning of the process; and because of the state of the chromatin, which in this condition of several vesicles, is entirely unlike the amitotic process of division.

In the same medusa from which Fig. 10 was taken there were

three other eggs in the same phase of maturation. *Pennaria* was collected by Professor Hargitt early in the morning and in the afternoon but in the numerous sections made of the adult medusæ, none showed any earlier stages. With this data at hand, however, it should be an easy matter to secure the intervening stages which undoubtedly occur about the time the medusæ are set free. Sections of medusæ still contained one or two eggs which in each instance had completed maturation, Fig. 11, and the polar bodies were not in contact with the egg. The conditions shown in Figs. 47 and 49 by Hargitt are without question polar bodies and probably the second. The state of the chromatin vesicles in Fig. 47 is more regular than any that I have seen which may be due to the preservation. They seem also to be more scattered than any observed in eggs fixed in Bouin. The clear area within the egg just beneath these vesicles was not seen in the Bouin fixation. Another puzzling feature of this study on maturation was the presence of small bodies attached to the surface of the egg. They were especially noticeable on the eggs of *Hydractinia*. After some study, it was apparent that they were protozoa which in many instances looked exactly like polar cells found in mollusca.

Chromatin Changes in *Pennaria*.

The female pro-nucleus is a small, faintly staining body that is found in eggs that are just laid and many that are still in the medusa. Sometimes it was pointed at the outer end and pushed close against the false membrane but it was never found protruding. It occupies this position until the approach of the male pro-nucleus when it may move some distance from the periphery of the egg. But before the fusion of the two pro-nuclei, there occurs a series of unusual changes in the chromatin, especially of the female pro-nucleus. Changes of a similar nature but not as extensive are found taking place in the male pro-nucleus. The chromatin changes described in *Hydractinia* preceded maturation. These in *Pennaria* follow maturation.

It is difficult to determine whether there is any order to these changes and so no attempt is made to decide which is the older state in the series of Figs. 12 to 18. In several of these figures

the male pro-nucleus is shown but its position, near or far from the female pro-nucleus does not seem to influence the time when the chromatin is to migrate from the nucleus. Immediately in contact with each pro-nucleus, the cytoplasm becomes denser and is composed of finer granules (cf. also Hargitt, Figs. 48, 49, 50). But isolated or wandering nuclear vesicles usually lack this modification of the cytoplasm which has been used to assist in recognizing the pro-nuclei.

Figs. 12, Pl. II., 15 and 18, Pl. III., show some of the chromatin granules close to the nuclear membrane as if they had just emerged from the nucleus into the cytoplasm. In both Figs. 15 and 18 these chromatin granules are on opposite sides of the nucleus which indicates that their position is not the result of the accident of cutting the sections. The male pro-nucleus in Fig. 15 shows the same condition of the chromatin. After the chromatin has been in the cytoplasm for some time, there are found definite small vesicles usually empty. From the study of mitosis in cleavage and the changes in the chromatin during the anaphase and telophase, the suggestion that these vesicles are the product of the transformed chromatin seems inevitable. During this period, while the chromatin is migrating into the cytoplasm, the chromatin both within and without the nucleus takes a very faint stain so that the whole nucleus is easily overlooked. Some of the most satisfactory results were obtained by using Brazilian without the iron mordant. In no instance have nuclei entirely devoid of chromatin been found. The vesicles in Figs. 13, 14, 17, Pl. II., and most of them in Fig. 12, are empty. If the interpretation offered is correct, then there must be a very large amount of chromatin that leaves the nucleus in Fig. 12. The meaning of the large flask-shaped vesicle attached to the female pro-nucleus in Fig. 15 is not understood.

There is some question as to whether this process takes place in all of the eggs preparatory to cleavage, but that it is very common and appears in well fixed eggs there can be no question. On the same slides were found mitotic figures preserved in all of their parts. The slides showing many of these phenomena were examined by Mr. George T. Hargitt who is at work on a similar problem at Harvard University and he confirmed the correctness

of these observations. The conditions certainly exist as drawn but more drawings could be made easily showing different relations of the chromatin masses and vesicles. The eggs just after deposition do not show these chromatin changes. After a time the chromatin that remains in the male and female pro-nuclei increases in amount and takes a deeper stain until the chromatin appears like that shown in Figs. 16, Pl. IV., and 19, Pl. III. Hargitt in Fig. 48 has two similar vesicles.

The vesicles last but a short time and usually are indistinguishable by the time that segmentation begins. But when such conditions as shown in Fig. 16 exist in the presence of a small vesicle containing chromatin near the female pro-nucleus and a second one between the two pro-nuclei, these remain longer in the egg. What their influence on subsequent development is, some light may be gained by a study of Fig. 49 (Hargitt). In this drawing there is a clearly defined mitotic figure with faint chromosomes; and a short distance away, three asters and their connecting fibers are around several vesicles. This Fig. 49 seems to be a later stage in the transformation of some of these vesicles containing chromatin and indicates a pseudo-segmentation in that it is not preceded by the fusion of the male and female pro-nuclei. It hardly seems as if such conditions played an important part in the future segmentation.

Fertilization in the Egg of *Pennaria*.

The spermatazoa penetrates the egg of *Pennaria* frequently before deposition, but the penetration is usual after the egg is laid. The false membrane may contain a large number of sperm heads that were unable to gain admission into the egg. The sperm head immediately after penetration becomes transformed into a vesiculate body, the male pro-nucleus. No definite place of entrance, nor path through the cytoplasm, nor the presence of asters could be determined. It remains smaller than the female pro-nucleus and is surrounded by fine cytoplasmic granules. Figs. 15, Pl. IV., 16, Pl. III., and 18, Pl. IV., give a good idea of the male pro-nucleus.

Figs. 17 and 19 show the approach of the pro-nuclei. The fine cytoplasmic granules surrounding the two bodies have united

in Figs. 15 and 19 and may have a stellate outline. It was practically impossible in Fig. 19 to determine whether there was a fusion at this stage of the two nuclei or whether they were merely in contact.

Polyspermia. — In Fig. 20, Pl. IV., there are shown conditions which seem to point strongly to polyspermia. The female pro-nucleus is present and some distance away a single vesicle which is similar in every respect to the male pro-nucleus of later stages. The cytoplasm is indented just opposite as if modified by the recent penetration of the sperm. Then close by this vesicle are nine other vesicles, somewhat smaller but otherwise identical with the single one. There is likewise the same modification of the cytoplasm just opposite. The natural explanation seems to be to regard these all as transformed sperm heads, and consequently polyspermia. Fig. 21, Pl. III., shows a second case of probable polyspermia where there are three nuclei, one much larger which is probably the female pro-nucleus. The other two are interpreted as male pro-nuclei. That several nuclei may exist previous to the first segmentation like this Fig. 21 and Fig. 48 of Hargitt there can be no question and that they are in some instances due to polyspermia I feel equally certain. But that this is the exclusive interpretation I do not accept because nuclear division may outrun cytoplasmic cleavage which results in giving several nuclei in the cytoplasm as Figs. 43 and 43*a* of Hargitt's clearly indicate and I have confirmed. It seems more probable that both explanations should be used in interpreting the multiple nuclear conditions.

Cleavage of the Egg in *Pennaria.*

This part of the paper on cleavage tends to show the manner of cleavage rather than a detailed description of the process as this has already been done by Hargitt. As was described in the section on fertilization, the two pro-nuclei approach preparatory to the first cleavage. Fig. 22, Pl. II., shows the prophase of the first cleavage. The female pro-nucleus has become much elongated and there are distinct asters at each end. The spindle fibers are just forming and the chromatin is still in the loose stage; the chromosomes are yet to form. The male pro-nucleus,

such of it as appears in the section, is near one end of the female pro-nucleus and much smaller. In Fig. 23 is shown another form of the prophase. This drawing was taken from the beginning of the second cleavage. The small prophase spindle lies entirely within the centrosphere and the chromosomes are in the form of vesicles. That these vesicles are modified chromosomes is proved by the condition of the chromosomes at the opposite pole of the old spindle where the process was not as advanced. Here some of the interzonal fibers were still in contact with the partly transformed chromosomes. The successive cleavage is so rapid in this instance that the new spindle has formed before the vesicles have united into a single vesicle, the nucleus. Nevertheless a normal spindle will result when the chromosome vesicles are transformed again into chromosomes. It is the study of such changes as these in the chromatin that convinced me that the vesicles in Fig. 10 were modified chromosomes and the vesicles in Figs. 12–16 were formed through the influence of chromatin astray in the cytoplasm. A third form of the prophase is quite common, Fig. 24, Pl. IV., where the nucleus is much elongated or somewhat irregular in outline. This prophase stage is farther advanced than the two previously described. The asters are larger and the forming spindle fibers more pronounced at each end. The chromatin has begun to take a deeper stain. In some respects all of these three forms of prophase are different, but the differences are not fundamental, and plenty of similar variations are known in other animals. They are all unmistakably by the mitotic process.

The metaphase is as typical as exists in any mitotically dividing cell. The chromosomes split and move to each pole, Figs. 23, 26, 27, Pl. II. During the late anaphase and early telophase the chromosomes become transformed into vesicles, Figs. 27, 23, may or may not unite into a single vesiculate nucleus before the next cleavage. I have been able to trace the nucleus continuously from its state in the unmaturated egg through all of the cleavage states. At no times does it dissolve other than in the normal mitotic changes. At no time does the total contents of the nucleus become dissipated throughout the cytoplasm to reform into separate nuclei. Other than the chromatin changes just

previous to segmentation, the nuclear phenomena in *Pennaria* appear perfectly normal.

Sometimes the cleavage is very regular as text-fig. 5 indicates, each cell shows the nucleus in the prophase and apparently so placed as to give off cells in the clockwise directions. The tilting of these nuclei and the perfect regularity is so apparent that this drawing might have been taken from an annelid or molluscan embryo, but in this same group of cells there was a most irregular embryo so that this regular condition is nothing more than an accident and is the only regular embryo among many sections.

The Centrosphere in the Egg of *Pennaria*.

It is difficult to decide on any of the old terms to describe the conditions in *Pennaria*. If the term centrosphere may include sphere and centrosome, the latter being potentially present only, then this may be taken as an acceptable term.

If there is a centrosome present in these eggs then it is an unstable body which varies greatly and is recognized with much difficulty. In all cases of the prophase, there remains a small clear area from which the fibers radiate but into which they do not penetrate. Fig. 24, Pl. III., shows a few fine granules in this area but their size, number, and staining reaction does not indicate that they are at all constant, nor can one detect that there is any inherent relation between these granules and rays or spindle fibers. That there is probably some substance that plays the part of a centrosome is apparent in Fig. 23, Pl. III., where the whole new spindle lies entirely within the old centrosphere. Therefore, it seems as if one might say that certain of these granules have potential centrosome properties and are possibly in the way to become differentiated; that is to say, that the elaborate centrosomes of mollusca for example indicate a higher degree of differentiation, while in these hydroids a similar result is produced by several granules which cannot be differentiated from the rest of the sphere substance.

The transformation of this centrosphere keeps pace with the changes in the chromosomes. By the time that the anaphase is reached the centrosphere substance has increased greatly in size,

Figs. 25, 26; and in late anaphase is very conspicuous, Fig. 27. After the metaphase stage, the astral fibers are mostly composed of granules, Figs. 25, 26. The interzonal fibers persist for some time and are frequently bent as the cleaving cytoplasm passes through them. At such times, the chromosomes lie at each end of a V-shaped figure. As the fibers disappear and the chromosomes become transformed into a nucleus, the centrosphere substance largely becomes an indistinguishable part of the cytoplasm, but a small portion remains as a clear, narrow region surrounding the nucleus. This means that the deeply staining, newly formed "resting nucleus" with a narrow transparent area around it in cleavage is perfectly normal, being the last remains of the centrosphere.

Papillæ in *Hydractinia* and *Pennaria*.

Hargitt (p. 469) has described these as ectosarcal phenomena. My study confirms his and shows that in both of these hydroids the papillæ are common in the unsegmented egg and have even been seen while the egg was in the medusa. There is no particular region on the egg where they arise. At first these papillæ were regarded as polar bodies, especially in *Hydractinia*, but when they were found on the vegetal pole and the female pro-nucleus was present at the animal pole in the same egg, such an interpretation was impossible. I believe that these papillæ are what Miss Bunting saw and described as polar cells, which is a mistake that might be easily made. In *Pennaria* these papillæ push the false membrane away from the egg as they form and after being set free remain in this same structure for some time. The papillæ in both species are wholly devoid of chromatin and so far as could be determined entirely cytoplasmic phenomena.

Fragmentation of the Nucleus, — Amitosis.

Fragmentation. — If by fragmentation of the nucleus is meant that the entire nucleus disappears and its contents disperse throughout the cytoplasm then I find no evidence of such a process in these hydroids. But what shall be said of the chromatin changes before maturation in *Hydractinia* and in *Pennaria* after maturation where large quantities of chromatin migrate into

the cytoplasm never to return to the nucleus so far as one can determine? This certainly seems to be a kind of fragmentation. Why does it occur? Has the maturation phenomena failed to fully prepare the egg nucleus for fertilization? These are questions which those who would make the development of all eggs conform to a definite series of changes must explain.

Amitosis. — By this process it is understood that the nucleus divides without the chromatin passing through a complicated series of changes and without the formation of a spindle. Frequent search has been made for amitosis in these eggs but without finding any positive evidence. The irregular and claviculate shaped nuclei were critically observed and in every instance eventually either asters or the very characteristic chromatin changes were taking place in them. The mere shape of the nucleus in *Pennaria* is no indication of amitosis, nor is it necessary that the chromosome vesicles become transformed into the single "resting nucleus." *The cluster of vesicles which Hargitt frequently finds is not uncommon in my material but is interpreted in this paper as late telophase.* This is a point concerning which there may be a difference of opinion but taking all of the facts into consideration, these vesicles seem to me to indicate mitosis rather than amitosis.

Inclusions in *Pennaria*.

There are found in the eggs of *Pennaria*, even before maturation, bodies which for the lack of a better term are designated inclusions, Fig. 29, Pl. IV. Thus far they have not been seen in the segmenting egg. As many as three such inclusions have been discovered in a single egg. When newly formed, the substance of the inclusion takes the same stain as the surrounding cytoplasm, but in the older stages this contained substance stains faintly, eventually leaving a cavity. This cavity then is obliterated by the encroachment of the cytoplasm. The substance within the inclusions in appearance and staining reactions is certainly similar, if not identical, to the cytoplasm, and the whole structure looks like a food-vacuole in which cytoplasm is being digested. Their origin has not been determined. They can hardly be regarded as polar bodies, because they may appear before maturation begins.

Summary.

1. In many particulars this work is a confirmation of the previous paper by Hargitt, especially in regard to the several nuclei in the unsegmented egg, the irregular shape of many such nuclei, and the irregular phases of cleavage.

2. The ova arise in any region of the polyp, which is contrary to Bunting's statements. The young ova gradually increase in size, during which time the nucleolus becomes vacuolated and the cytoplasm is occupied by numerous microsomes which become transformed into spherules. The cytoplasm changes its staining reaction during this time.

3. The chromatin during the growth of the egg stains less intensely than when in the younger state. Much of the chromatin migrates into the cytoplasm and is surrounded by vacuoles. The highly vacuolated condition of the cytoplasm is probably directly due to this migrating chromatin. The size of the nucleus decreases greatly.

4. In *Hydractinia* there were found three distinct kinds of granules, yolk masses, coarse granules and small bodies around the periphery. The small granules are distributed exclusively to the ectoderms.

5. Maturation begins in *Hydractinia* before the eggs are deposited. The process is by the formation of a distinct mitotic figure. It is very rare to find a polar body attached to the deposited egg.

6. The female pro-nucleus is very much smaller than the egg nucleus before maturation but it persists as a definite structure until cleavage begins. It is not at any time indistinguishable. The male pro-nucleus moves through the cytoplasm until it approaches the female pro-nucleus when the two fuse and fertilization is effected.

7. The first, and all subsequent cleavages, is by the mitotic process. A definite segmentation cavity is formed in the two-celled stage which increases in size. This cavity is gradually filled with cells until the planula is a solid mass of cells.

8. The false membrane in *Pennaria* is a transitory structure and probably of a fluid nature. Later its place is taken by a true membrane.

9. Maturation in *Pennaria* begins before the eggs leave the medusa. The polar bodies are ephemeral in character and rarely found attached to the deposited egg. The polar bodies are formed by the mitotic process.

10. After the two polar bodies are formed in *Pennaria* there is a distinct migration of a considerable amount of chromatin into the cytoplasm. The chromatin is transformed into vesicles which eventually are taken up by the cytoplasm. Sometimes these vesicles contain chromatin and persist for some time and may (?) divide mitotically, giving rise to a pseudosegmentation.

11. The spermatozoa may penetrate the egg before it is laid. The sperm head is transformed into a male pro-nucleus which moves through the cytoplasm toward the animal pole to unite with the female pro-nucleus.

12. Cleavage in *Pennaria* is at all times by the mitotic process. The chromosomes become transformed into vesicles during the late anaphase and early telophase. The vesicles may or may not unite into a definite "resting nucleus" before the next cleavage.

13. During cleavage in *Pennaria* there is a distinct centrosphere which contains granules with centrosome powers. This centrosphere is more conspicuous in *Pennaria* than in *Hydractinia*. The new prophase spindle arises within the old centrosphere.

14. Papillæ are found in both *Hydractinia* and *Pennaria* before segmentation, much as described by Hargitt.

15. A partial condition of fragmentation is seen in the migration of chromatin into the cytoplasm in both species.

16. No clear evidence of amitosis was observed.

17. Inclusions are frequently found in the egg of *Pennaria*.

October 20, 1908.

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POSTSCRIPT.

CHAS. W. HARGITT.

As supplemental to the foregoing paper it may not be amiss to add a few brief notes and comments. First, to the effect that it comprises an integral phase of work which has engaged the writer for many years, and which is still in progress. This particular feature was undertaken at my solicitation in the summer of 1906, as stated by the author. Second, at the same time I likewise turned over to my son, G. T. Hargitt, material for work along similar lines, a brief report of which has already been made. (*Science*, March 12, 1909.)

Smallwood's paper was completed nearly a year ago, but was at my request delayed, pending completion of further work of my own which was well advanced, and which it was intended should appear at the same time as intimately related thereto. The appearance of a brief note by Beckwith (BIOL. BULL., March, 1909), suggests the desirability of the issue of Smallwood's paper without further delay.

Aside from one or two points suggested by Beckwith I shall not undertake any detailed comments in this connection. Her rather matter-of-course dismissal of my presumed errors as to maturation with the remark that it was due "simply to the fact that eggs were not collected at the right time of day" is, to say the least, somewhat gratuitous! One does not usually follow in-

vestigations over a period of ten years without having taken *some* precautions against the more obvious sources of error. The fact is, I had long ago provided against that contingency. Again, her equally hasty dismissal of any question of methods of technique is without warrant. It was this more than any other one matter that proved an obstacle to satisfactory cytologic results. This I have called attention to in at least two of my more recent papers. And the precaution mentioned in the above paper by Smallwood as to this point was explicitly my own suggestion.

A brief comment as to the question of amitosis raised by both Smallwood and Beckwith must suffice for the *present.* In the first place I have never questioned the fact of mitosis in any of the cases under review, as the most cursory attention to my papers will show. Whether there be *amitosis* is purely a question of fact. Were my own results the only evidence it might very well be questioned. Facts adduced from almost every phylum of the animal kingdom are too well known at present to warrant further dogmatism on *a priori* or theoretical grounds. Whether my interpretation of the significance of the nuclear and chromatin fragmentation and the vesiculate "nuclear nests" may be warranted I shall defer for later consideration.

EXPLANATION OF PLATES.

EXPLANATION OF PLATE I.

FIG. 1. Egg nucleus with small amount of cytoplasm to show the migration of the chromatin. *Nl'*, nucleus; *Nll'*, nucleolus; *chr*, chromatin.

FIG. 2. Portion of the cytoplasm and nucleus. The chromatin is mostly in vacuoles. *Nl'*, nucleus; *chr*, chromatin.

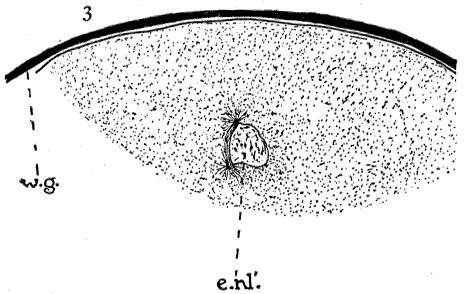
FIG. 3. Prophase first maturation. The walls of the nucleus are still intact. The egg is within the gonophore. *E. Nl'*, egg nucleus; *w. g*, wall of gonophore.

FIG. 4. Telophase of the second maturation. Egg deposited. *Cl. pol*^{2}, second polar body; *vsl. chr'so*, chromosome vesicles.

FIG. 5. Female pronucleus showing the remains of fibers that have persisted. Pronl. ♀, female pronucleus. *pr. gr*, peripheral granules.

FIG. 6. The approach of the male pronucleus. The female pronucleus is in the early prophase. Pronl. ♀, female pronucleus; pronl. ♂, male pronucleus.

FIG. 7. Prophase of third cleavage shows an elongated nucleus with asters and forming spindle fibers.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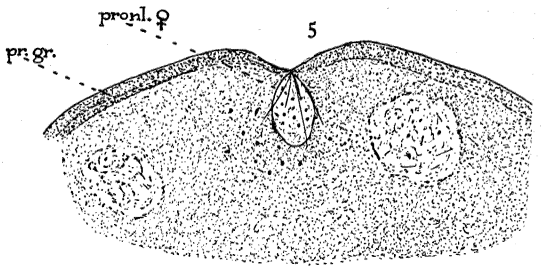
e.nl'.


7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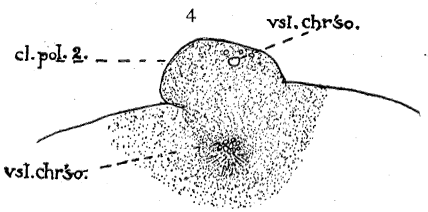
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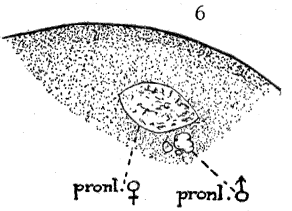
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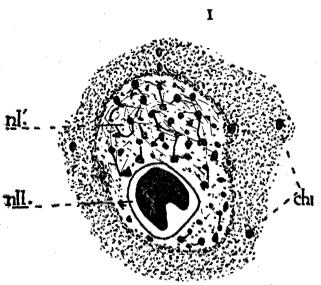
pronl.♂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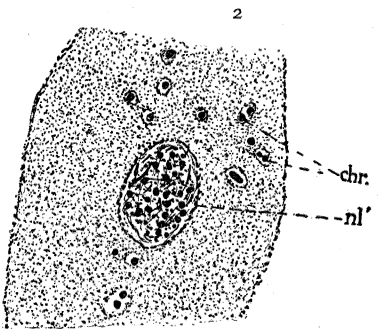
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nl'

Explanation of Plate II.

Fig. 8. Is taken from one of the cells shown in Fig. 9. The vacuolated condition of the cytoplasm is shown in the clear spaces. The spindle is in the anaphase and so directed as to set a cell free in the segmentation cavity. There is a large centrosphere at each pole.

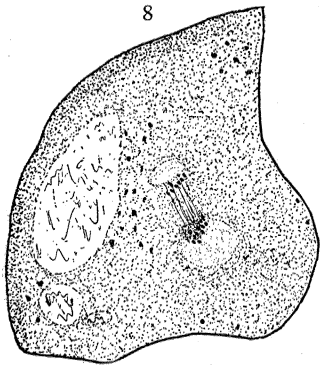
Fig. 9. Outline drawings of embryo. Numerous vacuoles are present in the cells. *Seg. cav*, segmentation cavity.

Figs. 10 to 29 are from *Pennaria.*

Fig. 10. Telophase second maturation. Egg in medusa. *Cl. pol. 1*, first polar body; *cl. pol. 2*, second polar body.

Fig. 11. Female pronucleus drawn from an egg before deposition. Pronl. ♀, female pronucleus.

Figs. 12 to 18 show the migration of the chromatin from the pronuclei into the cytoplasm. Pronl. ♀, female pronucleus; ♂, male pronucleus.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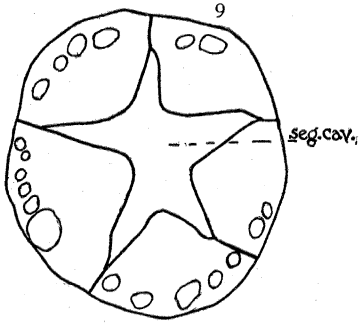
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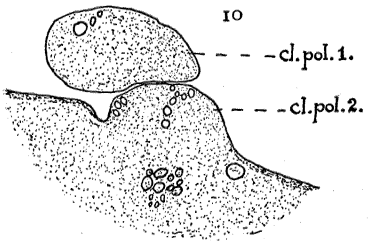
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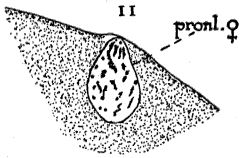
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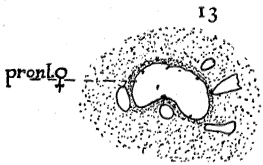
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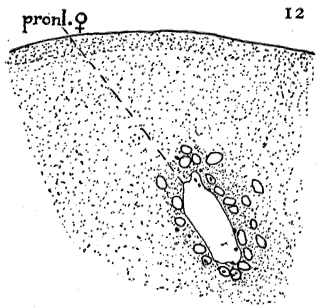
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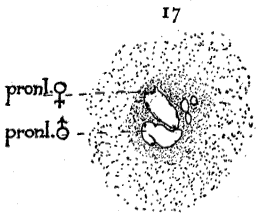
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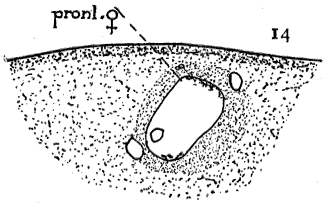
pronl.♂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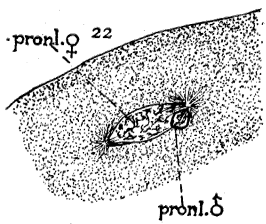
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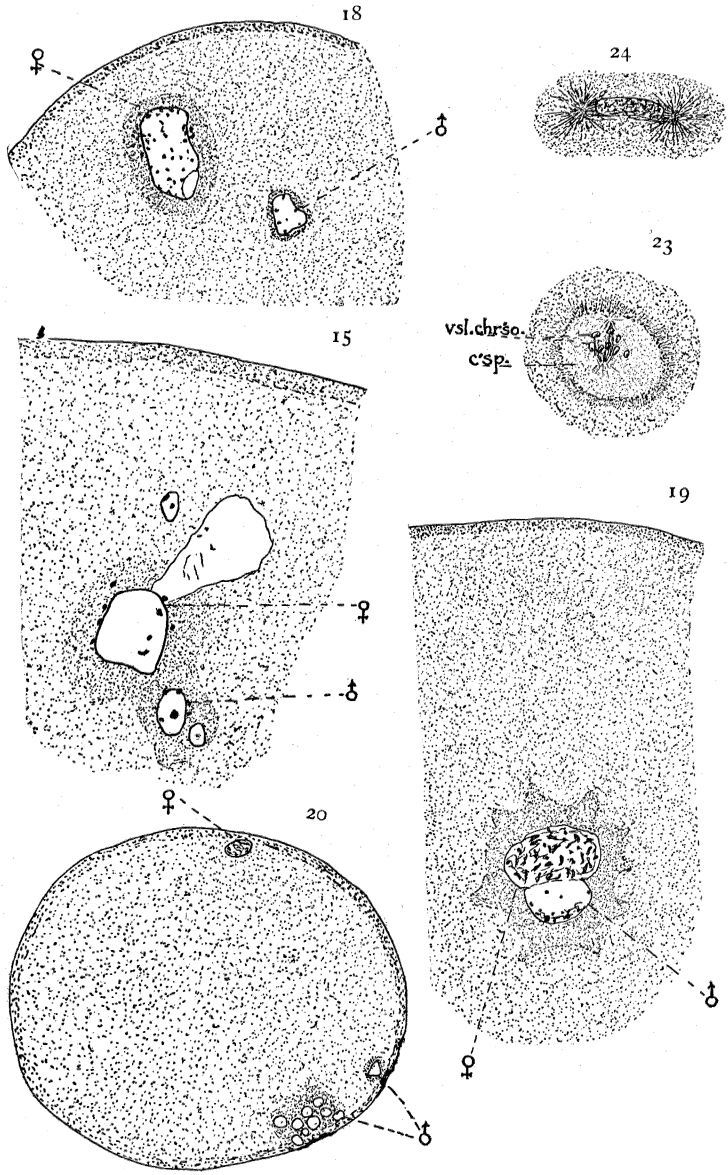
pronl.♂

Explanation of Plate III.

Fig. 19. Union of the male and female pronuclei. Pronl. ♀, female pronucleus; pronl. ♂, male pronucleus.

Fig. 20. Low power drawing of entire egg. The fine peripheral granules extend clear around the egg. Several nuclear-like bodies are interpreted as polyspermia.


18
24
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vsl.chrs.
c'sp.
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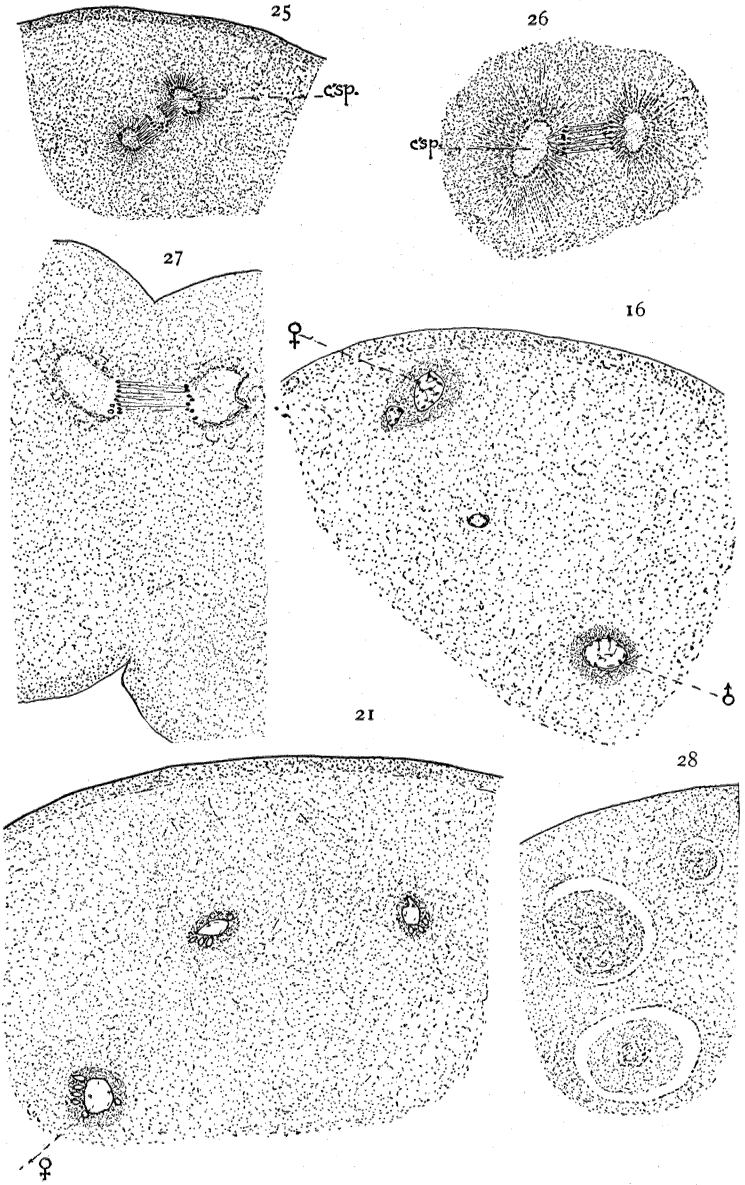
Explanation of Plate IV.

Fig. 21. Shows the presence of several nuclei in the unsegmented egg, one of which is probably the female pronucleus, pronl. ♀.

Figs. 22 to 24. Three forms of the prophase in cleavage. Fig. 22, conjugation of pronuclei and formation of first segmentation spindle. In Fig. 23 the chromosome vesicles have not united. Pronl. ♀, female pronucleus; pronl. ♂, male pronucleus; *vsl. chr'so*, chromosome vesicles; *c'sp*, centrosphere.

Figs. 25, 26, 27. Show the appearance of the segmentation spindle, migration of the chromosomes, and presence of the centrosphere; *c'sp*, centrosphere.

Fig. 28. Inclusions in the cytoplasm.


25
csp.
26
csp.
27
16
♀
♂
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28
♀